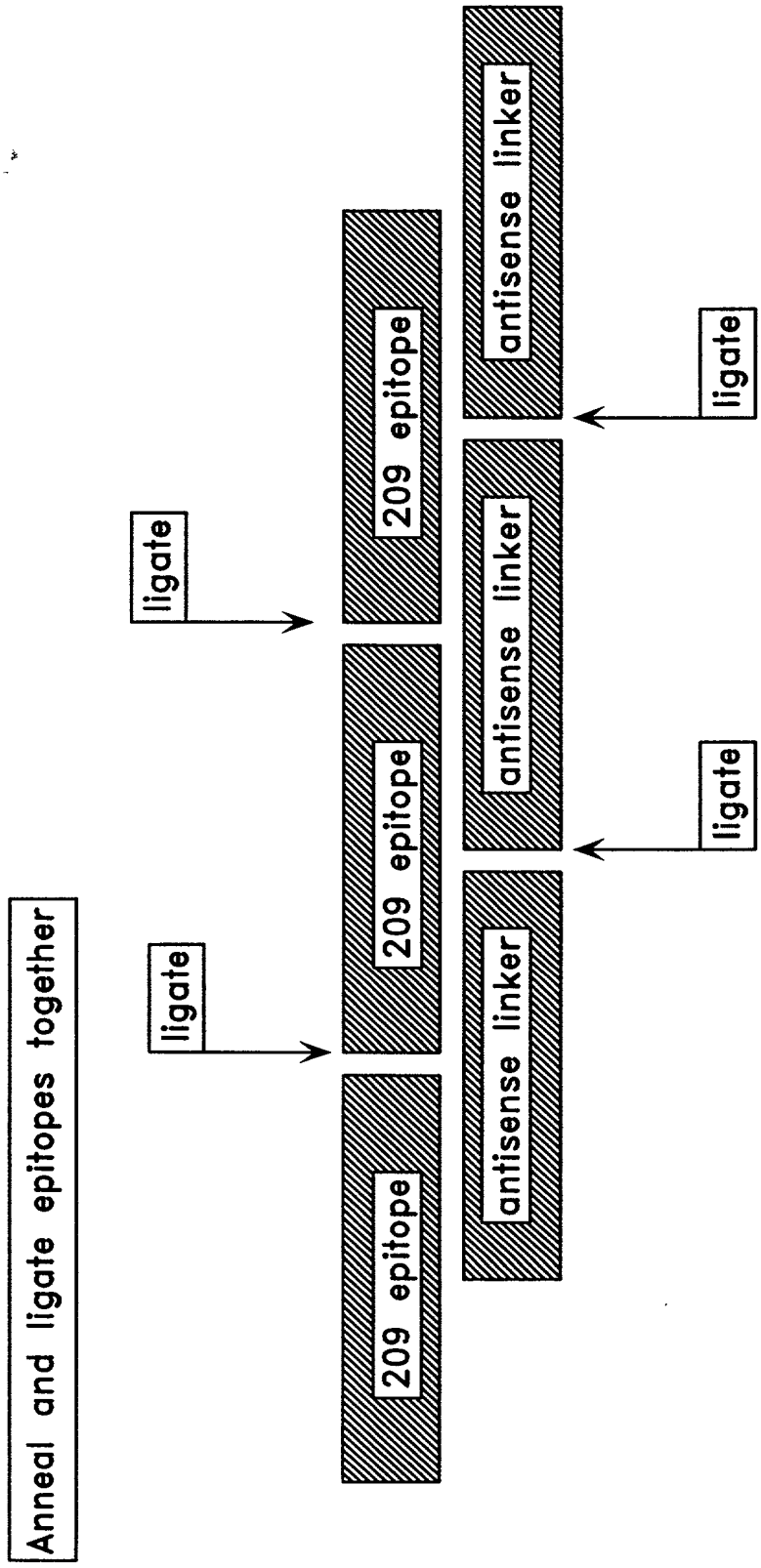

Anneal and ligate epitopes together
ligate
ligate
ligate
209 epitope
209 epitope
209 epitope
antisense linker
antisense linker
antisense linker

Fig. 1

Structure of gp100–209 epitope concatamer

Concatamer of Gp100–209 epitope was generated using the following strategy.

Concatomer Primers

1. GP100–209 forward

5'
ATTACTGACC AGGTACCTTT CTCCGTG

2. GP linker

5'
TGGTCAGTAA TCACGGAGAA AGGTACCT

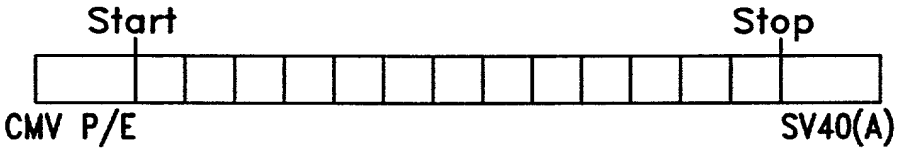
3. Start primer (Eco RV) 30 mer 46% GC

5'
GGCC GATATC ATGATTACTG ACCAGGTACC

4. Stop Primer (Spe) 30 mer 53% GC

5'
GGCC ACTAGT GATCACGGAG AAAGGTACCT

Structure of gp100–209 epitope concatamer expression cassette

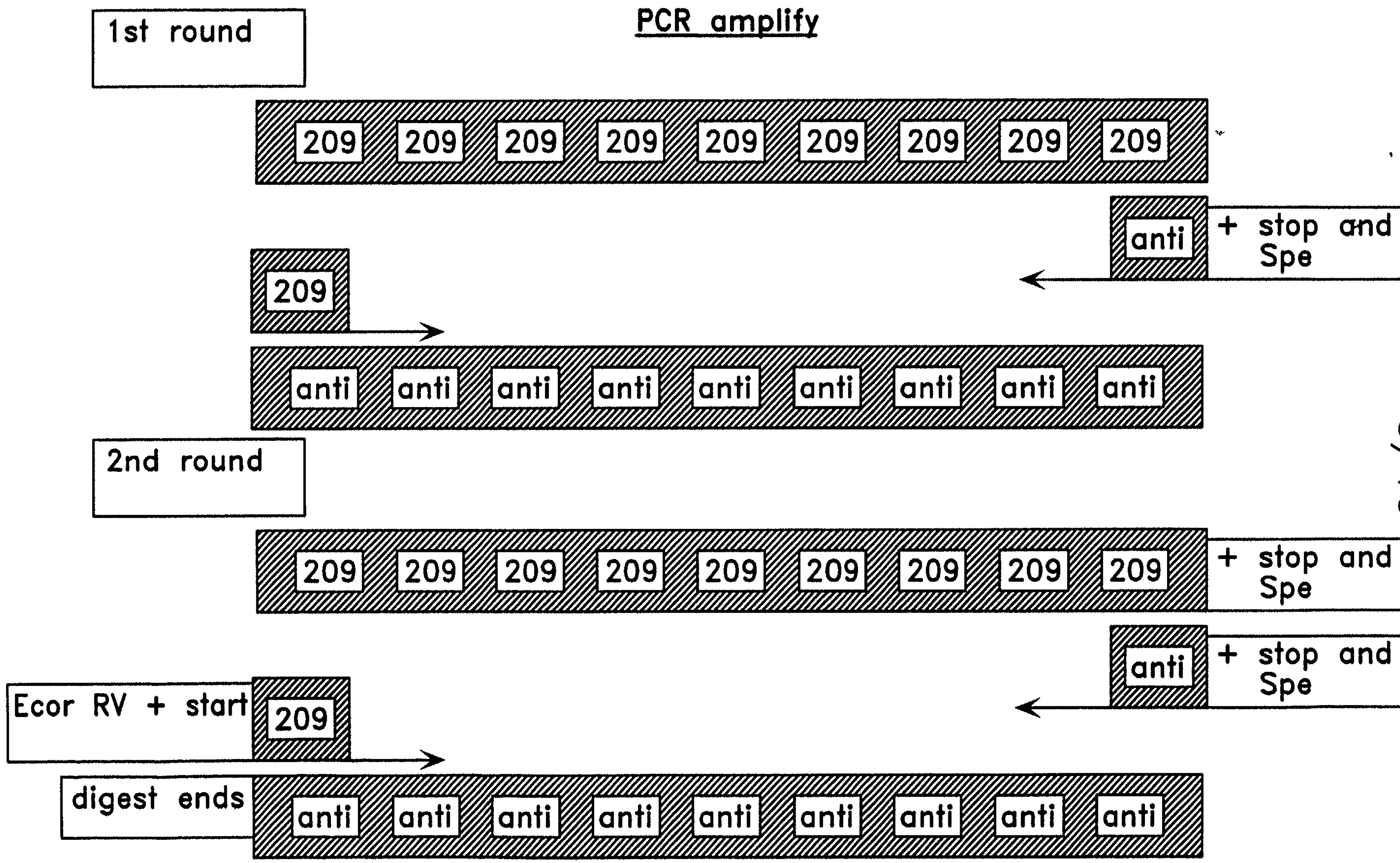


13 repeats of gp100–209 epitope

Gp100–209 epitope sequence

5' ATTACTGACCAGGTACCTTTCTCCGTG 3'
3' TAATGACTGGTCCATGGAAAGAGGCAC 5'

Fig. 2A



PCR amplify
1st round
209
anti
+ stop and Spe
2nd round
Ecor RV + start
digest ends

Fig. 2B

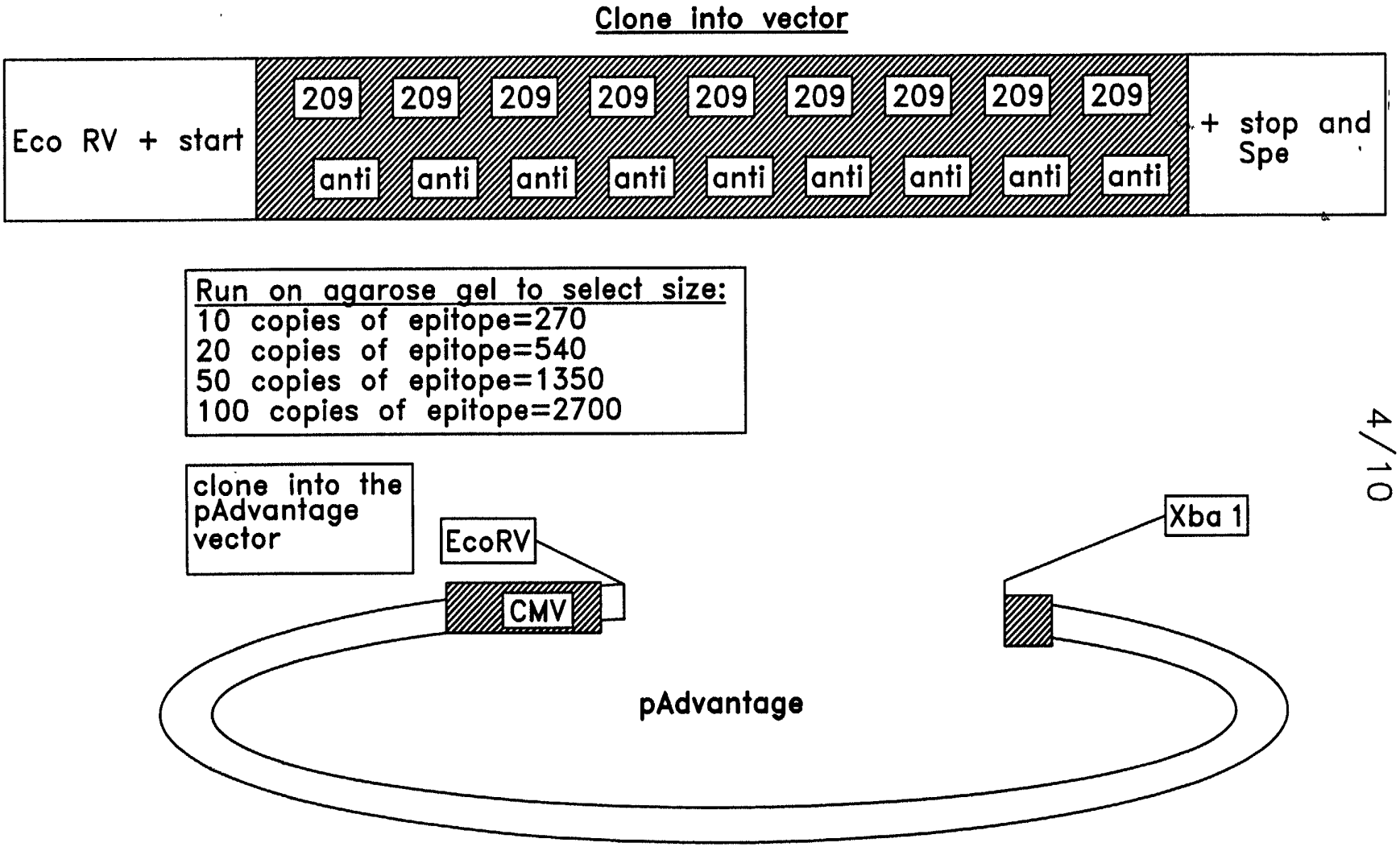

Clone into vector
Eco RV + start
209
anti
+ stop and Spe
Run on agarose gel to select size:
10 copies of epitope=270
20 copies of epitope=540
50 copies of epitope=1350
100 copies of epitope=2700
clone into the pAdvantage vector
EcoRV
CMV
Xba 1
pAdvantage

Fig. 2C

gp209 epitope construct

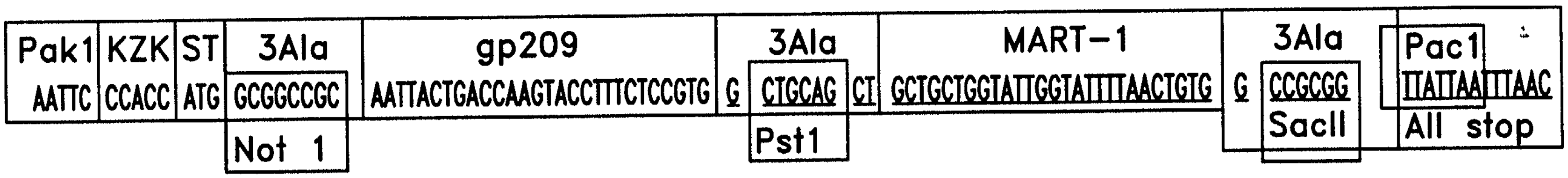


Fig. 3A

α-globin stability element

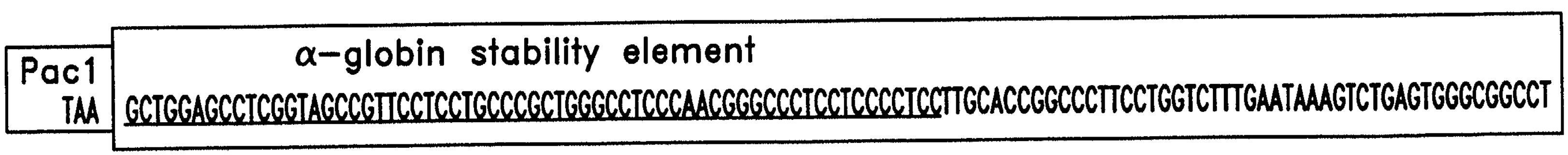


Fig. 3B

Fig. 4

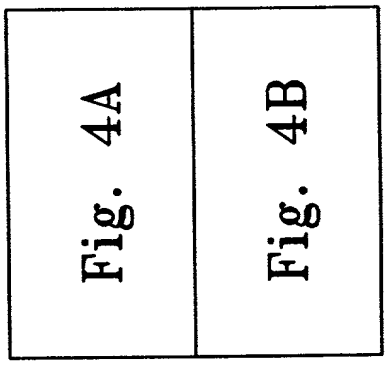


Fig. 4A

```
              start
              |    10         20         30         40         50         60         70         80
              v*   *     *    *     *    *     *    *     *    *     *    *     *    *     *    *
1350 9 cop    ATGATTACTG ACCAGGTACC TTTCTCCGTG ATTACTGACC AGGTNCCTTT CTCCGTGATT NCTGACCAGG TNCCTTTCTC
              ____560__a____570__a____580__553 TO 558 OF 560 2 F.a____610__a____620__a____630_>
                          10         20
jmpl str +      ATTACTG ACCAGGTACC TTTCTCCGTG>
                ||||||| |||||||||| ||||||||||
1350 9 cop      ATTACTG ACCAGGTACC TTTCTCCGTG
                                             10         20
jmpl str +                           ATTACTGACC AGGTACCTTT CTCCGTG>
                                     |||||||||| |||| ||||| |||||||
1350 9 cop                           ATTACTGACC AGGTNCCTTT CTCCGTG
                                                                          10         20
jmpl str +                                                        ATT ACTGACCAGG TACCTTTCTC>
                                                                  |||  ||||||||| | ||||||||
1350 9 cop                                                        ATT NCTGACCAGG TNCCTTTCTC
                    90         100        110        120        130        140        150        160
                 *   *     *    *     *    *     *    *     *    *     *    *     *    *     *    *
1350 9 cop    CGTGATTNCT GACCAGGTNC CTTTCTCCGT GATTNCTGAC CAGGTNCCTT TCTCCGTGAT TNCTGACCAG GTNCCTTTCT
              _____640__a_____650__a____660__553 TO 558 OF 560 2 F.a_____690__a_____700__a_____710_>
jmpl str +    CGTG>
              ||||
1350 9 cop    CGTG
                          10         20
jmpl str +      ATTACT GACCAGGTAC CTTTCTCCGT G>
                ||| || |||||||| | |||||||||| |
1350 9 cop      ATTNCT GACCAGGTNC CTTTCTCCGT G
                                             10         20
jmpl str +                           ATTACTGAC CAGGTACCTT TCTCCGTG>
                                     ||| ||||| ||||| |||| ||||||||
1350 9 cop                           ATTNCTGAC CAGGTNCCTT TCTCCGTG
                                                                          10         20
jmpl str +                                                        AT TACTGACCAG GTACCTTTCT>
                                                                  || | |||||||| || |||||||
1350 9 cop                                                        AT TNCTGACCAG GTNCCTTTCT
```

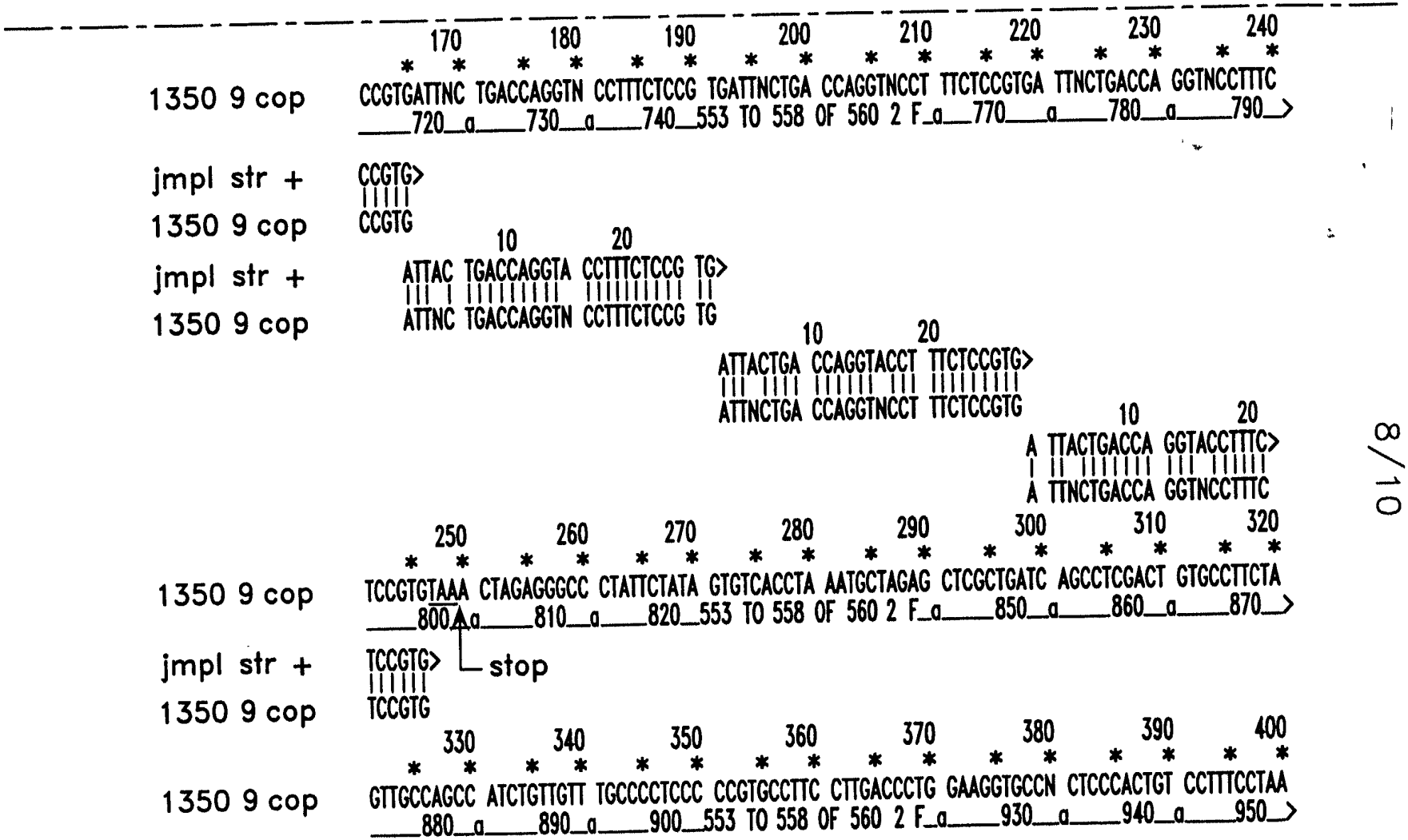

170 180 190 200 210 220 230 240
1350 9 cop CCGTGATTNC TGACCAGGTN CCTTTCTCCG TGATTNCTGA CCAGGTNCCT TTCTCCGTGA TTNCTGACCA GGTNCCTTTC
720 730 740 553 TO 558 OF 560 2 F 770 780 790
jmpl str + CCGTG>
1350 9 cop CCGTG
10 20
jmpl str + ATTAC TGACCAGGTA CCTTTCTCCG TG>
1350 9 cop ATTNC TGACCAGGTN CCTTTCTCCG TG
10 20
ATTACTGA CCAGGTACCT TTCTCCGTG>
ATTNCTGA CCAGGTNCCT TTCTCCGTG
10 20
A TTACTGACCA GGTACCTTTC>
A TTNCTGACCA GGTNCCTTTC
250 260 270 280 290 300 310 320
1350 9 cop TCCGTGTAAA CTAGAGGGCC CTATTCTATA GTGTCACCTA AATGCTAGAG CTCGCTGATC AGCCTCGACT GTGCCTTCTA
800 810 820 553 TO 558 OF 560 2 F 850 860 870
jmpl str + TCCGTG> stop
1350 9 cop TCCGTG
330 340 350 360 370 380 390 400
1350 9 cop GTTGCCAGCC ATCTGTTGTT TGCCCCTCCC CCGTGCCTTC CTTGACCCTG GAAGGTGCCN CTCCCACTGT CCTTTCCTAA
880 890 900 553 TO 558 OF 560 2 F 930 940 950

Fig. 4B

T cell mediated Lysis of gp100-209 epitope presenting cells

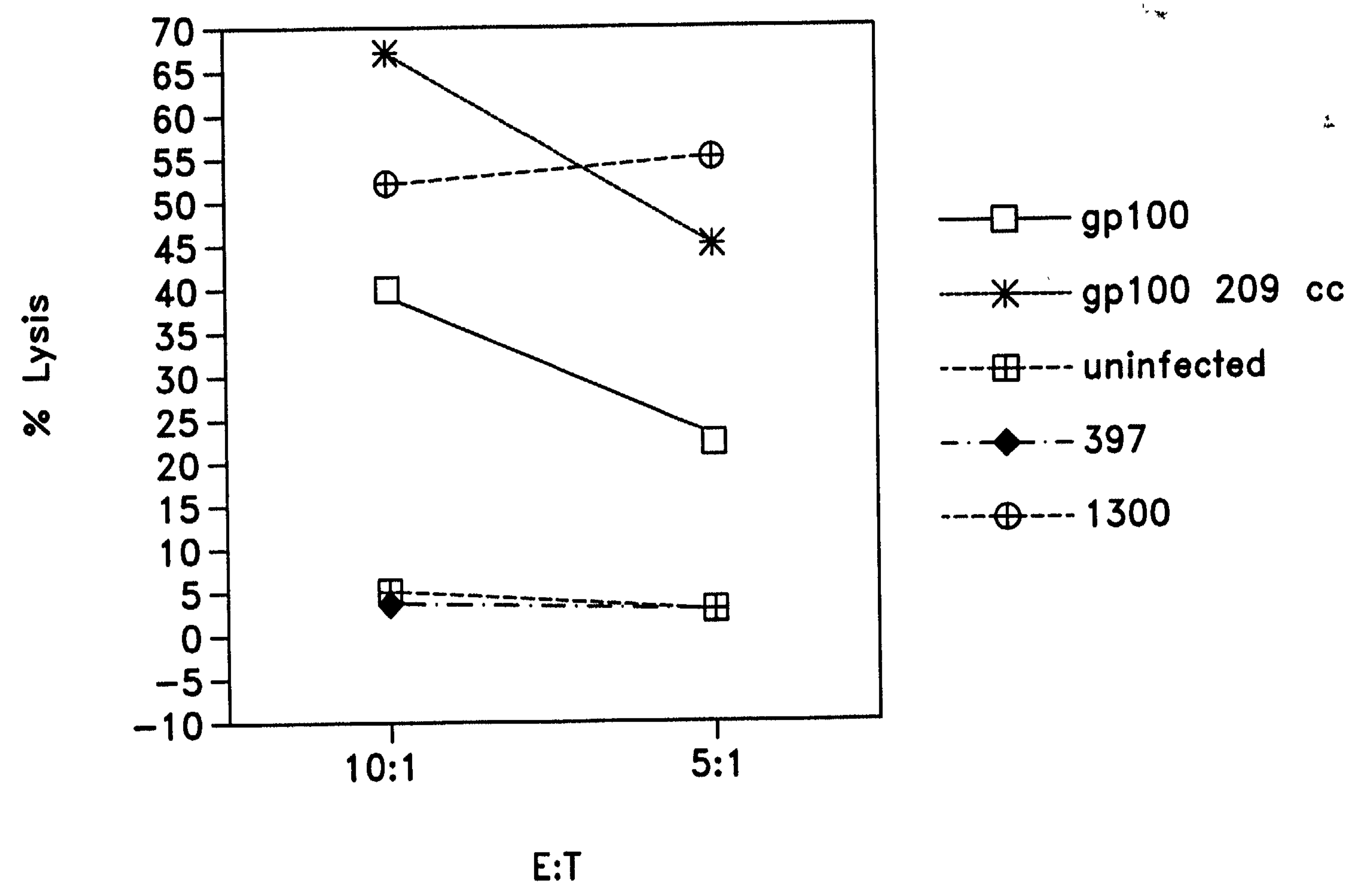


Fig. 5A

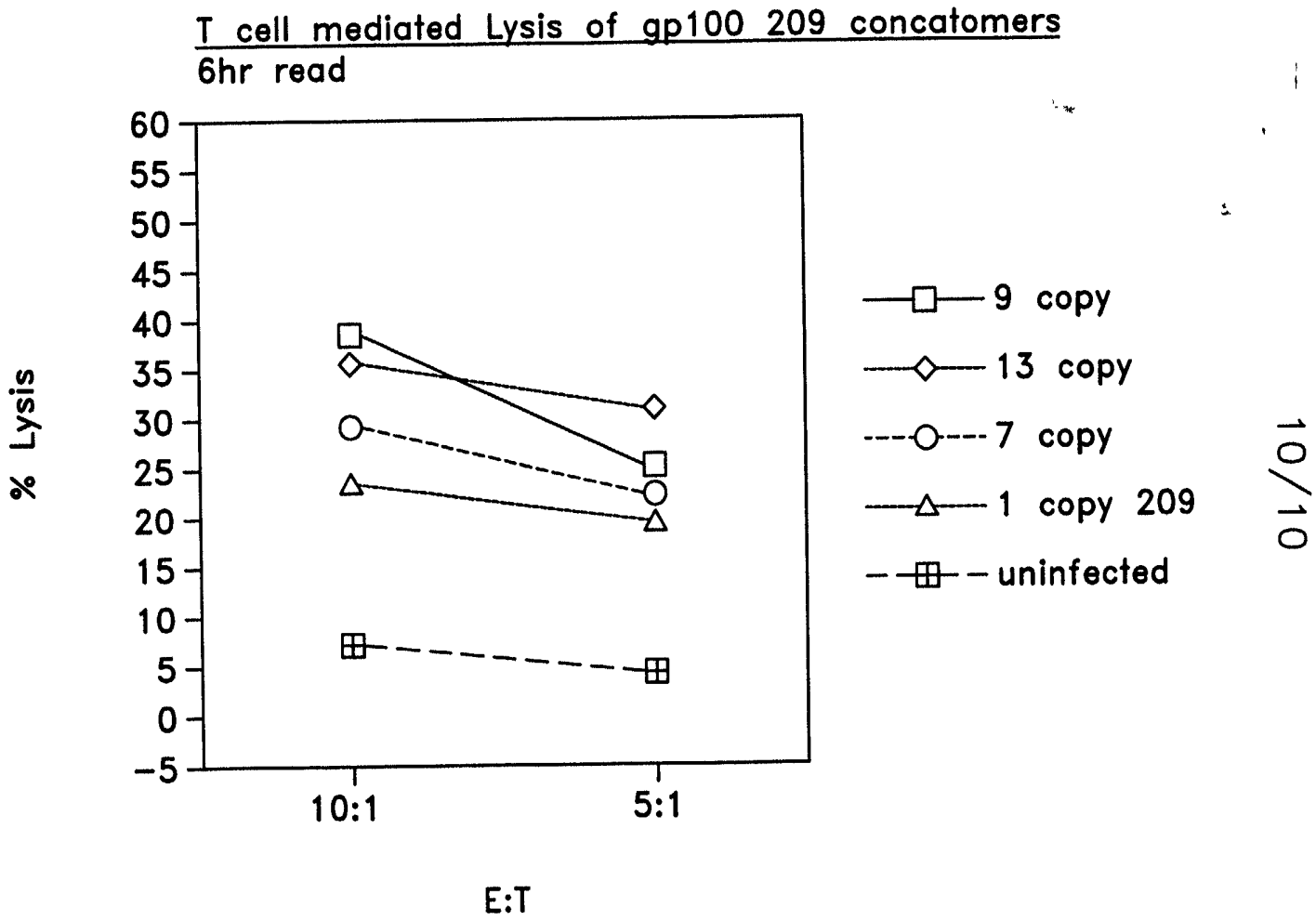

T cell mediated Lysis of gp100 209 concatomers
6hr read
% Lysis
60
55
50
45
40
35
30
25
20
15
10
5
0
-5
10:1
5:1
E:T
9 copy
13 copy
7 copy
1 copy 209
uninfected

Fig. 5B